TESTING OF GREAT BAY OYSTERS FOR TWO PROTOZOAN PATHOGENS

A Final Report to

The New Hampshire Estuaries Project

Submitted by

New Hampshire Fish and Game Department

May 21, 2003

This report was funded in part by a grant from the Office of State Planning, New Hampshire Estuaries Project, as authorized by the U.S. Environmental Protection Agency pursuant to Section 320 of the Clean Water Act.

New Hampshire Estuaries Project

TESTING OF GREAT BAY OYSTERS FOR TWO PROTOZOAN PATHOGENS

Table of Contents

Executive Summary

Two protozoan pathogens, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) are known to be present in Great Bay oysters. With funds provided by the New Hampshire Estuaries Project (NHEP), the Marine Fisheries Division of New Hampshire Fish and Game Department, (NHF&G) has continued assessing the presence and intensity of both disease conditions in oysters from the major beds within the Great Bay estuarial system.

Introduction

The American oyster, *Crassostrea virginica,* may be invaded by a variety of parasites. Two particularly damaging protozoan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), have caused widespread high mortalities along the Southern and Middle Atlantic Coast and are now found in New Hampshire waters.

MSX was first recognized as a serious oyster pathogen in Delaware Bay in 1957 (Haskin and Andrews, 1988). It has since spread to the degree that it now is reported from Florida to Maine. The presence of MSX in New England was first noted in 1960 from oysters taken at Milford, Connecticut (Sindermann and Rosenfield, 1967). In 1967, oysters from Wellfleet, Massachusetts were found to contain MSX ((Krantz et al, 1972). The presence of MSX in the Piscataqua River oysters was first established in 1983 although an unspeciated haplosporidian plasmodia was seen by Maine Department of Marine Resource scientists in 1979 (S. Sherburne, Maine Department of Marine Resources, per com.). Following this, MSX is not recorded again until 1994 when a Maine based aquaculture operation, Spinney Creek Shellfish, Inc., found Piscataqua River specimens contained MSX. Oysters from these same beds were examined a year later (1995) and again MSX was found, this time in higher prevalence than the previous year (Ken LaValley, Spinney Creek Shellfish, Inc., per com.).

In response to the Spinney Creek Shellfish, Inc. test results and to anecdotal information from New Hampshire recreational oyster harvesters of many boxed and/or gaping oysters, three major New Hampshire Great Bay beds were sampled and tested in 1995.. This initial histological examination of samples was done by Dr. Bruce Barber, University of Maine. In later years, tests have been done by the Haskins Shellfish Research Laboratory. Results of all MSX tests are covered below.

Dermo has spread from South and Middle Atlantic sources up the coast and into the Gulf of Maine during the past three decades. North of Chesapeake Bay, cold waters are believed to act as a controlling factor that prevents year-round persistence of Dermo, making its virulence to oysters in New England waters probably minor compared to MSX. Dermo was first demonstrated to be present in the Great Bay system in 1996. Oysters from Spinney Creek, a small tidal pond off the Piscataqua River, were seen to harbor Dermo when examined by University of Maryland scientists. Following this, samples were taken from Great Bay and the Piscataqua River, and these showed Dermo-like particles. Dermo tests from Great Bay system specimens will be reviewed in greater detail below.

Project Goals and Objectives

It appears, based on recent oyster abundance monitoring and from the information gleaned by survey of oyster harvesters, that the last decade has been a period of oyster abundance drop and harvest decline. It is quite likely the presence of both MSX and Dermo has contributed to recent declines in the Great Bay oyster stock. It is important to maintain some surveillance of these disease conditions as the presence or absence of such potentially damaging pathogens may help explain future oyster abundance variability. The objective of this study is to monitor the presence of MSX and Dermo in Great Bay oysters**.**

Methods

Oysters, were collected from four Great Bay system areas in the fall of 2002. Sample locations were Adams Point, Nannie Island, Oyster River and Salmon Falls River (Fig. 1). NOTE- FIGURE 1 (PAGE 3 OF THE REPORT) IS NOT AVAILABLE ELECTRONICALLY. Except for the imported "disease resistant" oysters taken from the Salmon Falls, all were 65mm shell length or greater. The smaller "disease resistant" test subjects were 3 year oysters. These samples were sent to testing laboratories at Rutgers University/Haskins Shellfish Research Laboratory (Susan Ford).

MSX determinations were accomplished by tissue section histology. They were processed using standard techniques and examined microscopically for pathological conditions or parasites, particularly MSX.

Dermo testing involved the standard Ray's fluid thioglycollate medium incubation of rectal and mantle tissues.

Results and Discussion

The results of all recent tests for MSX, 1995 to present, are shown in Table 1. Dermo results for the past seven years of testing are shown in Table 2.

The MSX results in general, over the eight years of testing, show a widespread distribution of infection throughout the Great Bay system. Levels of prevalence vary site to site and within sites over time. It appears, based on early test results, that the Piscataqua River area was most severely impacted by the 1995 epizootic (Barber et al 1997). Systemic infections in the upper reaches of the Piscataqua River and Salmon Falls River ranged from 25% to 50% compared to generally lower values in Great Bay proper (Table 1.). An exception to this general pattern is shown in the 1997 Nannie Island data that show relatively high values for both numbers infected and number of systemic infections. The year 2002 tests show some lessening of the more lethal systemic infections but a continued presence of MSX throughout the Great Bay

system. For now, it is clear that MSX exists at all locations where oysters have been sampled in the Great Bay system.

Early Dermo results show the presence of Perkinsus-like particles at all locations sampled except for Seal Rock, Fox Point and Bellamy River. All except the Sturgeon Bed and Piscataqua

Table 1. MSX Test Results

Date	Location	No. Tested	No. Infected [1]	No. Systemic Infection [1]
9/05/95 [2]	Piscataqua River (Summer Bed)	25	18 (72%)	10 (40%)
10/27/95 [2]	Salmon Falls	16	13 (81%)	8 (50%)
10/27/95 [2]	Piscataqua River (Summer Bed)	20	14 (70%)	5 (25%)
10/27/95 [2]	Sturgeon Bed	20	13 (65%)	8 (40%)
10/27/95 [2]	Stacy Bed (Seal Rock)	20	9 (45%)	2 (10%)
11/06/95	Adams Point	20	8 (40 %)	3 (15%)
11/06/95	Nannie Island	20	3 (15%)	1 (5%)
12/18/95	Oyster River	20	10 (50%)	6 (30%)
4/12/96	Nannie Island	30	3 (10%)	0
5/27/96	Adams Pt.	10	0	0
5/27/96	Nannie Island	10	0	0
3/17/97	Fox Pt.	30	5 (16.6%)	1 (3.3%)
9/08/97	Bellamy River	25	10 (40%)	2 (8%)
9/08/97	Squamscott River	25	11 (44%)	5 (20%)
11/17/97	Adams Point	25	10 (40%)	5 (20%)
11/17/97	Nannie Island	25	13 (52%)	7 (28%)
11/17/97	Oyster River	25	9 (36%)	2 (8%)
11/17/97	Piscataqua River	25	15 (60%)	5 (20%)
12/9/98	Adams Point	25	7 (28%)	2 (8%)
12/9/98	Nannie Island	25	11 (44%)	2 (8%)
12/9/98	Squamscott River	25	17 (68%)	7 (28%)
12/9/98	Piscataqua River	18	7 (39%)	3 (11%)
10/21/99	Nannie Island	20	7 (35%)	6 (30%)
11/4/00	Piscataqua River	20	6 (30%)	3 (15%)
11/4/00	Adams Point	20	7 (35%)	5 (25%)
11/4/00	Nannie Island	20	6 (30%)	5 (25%)
11/15/00	Oyster River	20	7 (35%)	2 (10%)
10/10/01	Nannie Island	24	5 (21%)	4 (17%)
10/18/01	Salmon Falls - disease resistant	20	1 (5%)	1 (5%)
01/18/01	Salmon Falls - native	21	9 (43%)	6 (29%)
11/4/01	Oyster River	20	5 (25%)	4 (20%)
11/4/01	Adams Point	20	5 (25%)	4 (20%)
10/14/02	Oyster River	20	9 (45%)	1 (5%)
10/14/02	Adams Point	20	9 (45%)	0
10/20/02	Salmon Falls - disease resistant	20	2 (10%)	0
10/20/02	Salmon Falls - natives	18	5 (28%)	0
10/31/02	Nannie Island	24	9 (37%)	4 (17%)

1) Presence of MSX plasmodia when found in palps and gills only are recorded as infections only. When plasmodia are found in tissue other than palps and gills (i.e. digestive gland, haemolymph, gonads) the infection is considered systemic.

2) Data from Barber et al 1997.

Table 2. DERMO Test Results

Date	Location	No. Tested	No. Oysters in each infection category[1)] 0.5	1	2	3	4	5	Prevalence
12/16/96	Nannie Island	25	1						4%
12/16/96	Seal Rock	25	0	0	0	0	0	0	0
12/16/96	Sturgeon Bed	25	2				1		12%
3/17/97	Fox Pt.	30	0	0	0	0	0	0	0
8/14/97	Piscataqua River	25	2	2			1		20%
8/17/97	Adams Pt.	25	4						16%
8/14/97	Oyster River	25	1						4%
8/14/97	Nannie Island	25	1						4%
9/08/97	BellamyRiver	25	0	0	0	0	0	0	0
9/08/97	Squamscott River	25	1						4%
11/17/97	Adams Pt.	25	1						4%
11/17/97	Nannie Island	25	0	0	0	0	0	0	0
11/17/97	Oyster River	25	0	0	0	0	0	0	0
11/17/97	Piscataqua River	25	0	0	0	0	0	0	0
12/9/98	Adams Pt.	25	0	0	0	0	0	0	0
12/9/98	Nannie Island	25	0	0	0	0	0	0	0
12/9/98	Squamscott River	25	0	0	0	0	0	0	0
12/9/98	Piscataqua River	18	0	0	0	0	0	0	0
10/21/99	Nannie Island	20	0	0	0	0	0	0	0
11/4/00	Piscataqua River	20	0	0	0	0	0	0	0
11/4/00	Adams Pt.	20	0	0	0	0	0	0	0
11/4/00	Nannie Island	20	0	0	0	0	0	0	0
11/15/00	Oyster River	20	0	0	0	0	0	0	0
10/10/01	Nannie Island	25	0	0	0	0	0	0	0
10/18/01	Salmon Falls (disease resistant)	25	3	0	0	0	0	0	12%
10/18/01	Salmon Falls (native)	25	6	5	1	1	1	1	60%
11/4/01	Oyster River	20	0	0	0	0	0	0	0
11/4/01	Adams Point	20	0	0	0	0	0	0	0
10/14/02	Adams Point	20	1	2	0	0	0	0	15%
10/14/02	Oyster River	20	0	0	0	0	0	0	0
10/31/02	Nannie Island	24	2	0	0	0	0	0	8%
11/20/02	Salmon Falls (native)	18	4	2	1	1	1	2	61%
11/20/02	Salmon Falls (crossbreeds)	20	1	0	0	0	0	0	5%

1) Infection categories are based on the severity of infection. Categories 0.5 to 2 are generally thought of as light or minor, whereas categories 3 to 5 are moderate to heavy and may pose an infection threat to Dermo-free oysters.

River sites are light infections that appear to show low prevalence. The year 2002 Dermo results, unlike those of 1998, 1999, 2000 and 2001 reveal a reoccurrence of Dermo at all sites except Oyster River. Notable points relative to the 2002 data are as follows:

- DERMO continues to be found in greatest prevalence at the Salmon Falls bed with natives more infected than crossbreeds. Crossbreeds are all young oysters (3 yr) while natives are older. Only at the Salmon Falls bed does one find advanced stages of infection which may pose an infection threat to DERMO-free oysters.
- Nannie Island shows minor infections for the first time since 1997 (five years DERMO-free). The same can be said for Adams Point except that there was no 1999 DERMO testing there.
- Oyster River is the only site free of DERMO in 2002. DERMO was found there only in the 1997 testing, however, yearly tests are not available for this site.

With the 2002 test information now available one can conclude that Dermo is widely distributed but of low prevalence throughout the oyster stock of the Great Bay system. It is noteworthy that the principal recreational harvest beds, Nannie and Adams Point, that had been Dermo-free over the past four years now show DERMO again. The upper Salmon Falls River area is the only area where high infection levels are found.

The most recent test information (year 2002) shows MSX presence at all four locations sampled. Except for the Salmon Falls River, the percent of infected oysters is about the same for all areas (i.e., 37-45%). The Salmon Falls presents an interesting picture with putative disease resistant test subjects showing very low infection and natives higher infection rates. Systematic infection involving the presence of plasmodia in tissues other than palps and gills (i.e., digestive gland, haemolymph and gonads) is variable throughout the Great Bay system. Only two sites, Nannie Island and the Oyster River show some systemic infections. Only one location (two oysters) showed evidence of advanced systemic infection. These advanced levels of infection generally prove lethal.

The year 2002 MSX test results, compared with those of earlier years show continued presence of MSX but some reduced level of serious systemic infection. The infection levels of the crossbreed ("disease resistant") oysters at the Salmon Falls bed continue to be lower than the natives at the same location. Because these "disease resistant" oysters are smaller (i.e., younger) than the natives, it would be unreasonable to make direct comparisons with natives at this time. The promise of a true disease-resistant oyster remains unproven by the results reported herein. Future testing of both native and disease-resistant oysters of similar age (and exposure history) will be necessary to evaluate differential MSX resistance.

Conclusions

Evidence of a large scale oyster mortality within Great Bay Estuary first gained regional attention in the fall of 1995. This prompted examination of oyster from several New Hampshire oyster beds. Results of these examinations focused on the presence of *Haplosporidium nelsoni* (MSX), an oyster pathogen well known to the middle Atlantic oyster grounds as a cause of epizootics.

During this same time, the Piscataqua and Salmon Falls River beds in Maine waters were the sites of similar oyster MSX mortality (Ken LaValley, Spinney Creek Shellfish, Inc., per. com.). The 1995 Great Bay Estuary MSX epizootic caused over 80% mortality in the areas most affected (Barber et al 1997). Highest mortalities were found in the Piscataqua and Salmon Falls Rivers.

It is important to note that no testing specific for Dermo was done immediately following the reported fall 1995 oyster mortality.

In 1996 spring testing at the major New Hampshire recreational oystering beds; Nannie Island and Adams Point, showed no systemic infections of MSX. The 1996 season did not result in oyster mortalities of the type observed in the previous year. In 1997, 1998, 2000 ,2001and 2002 monies from NHEP were received to support a more expansive testing program for both MSX and Dermo.

Based on tests performed annually since 1995, we know two protozoan parasites (ie, MSX and Dermo) are widely distributed within the Great Bay oyster stock. Severity of infection and prevalence vary from site to site and over time at a specific site.

The year 2002 oyster tests show continued presence of MSX at all beds. Dermo was seen, after a near five year absence in oysters from all beds except the Oyster River.

Recommendations

- This testing program should continue with at least yearly samples from all major oyster beds within the Great Bay system.

- Movement of oysters from bed to bed within the Great Bay system should be discouraged as it may lead to distribution of infective stages of Dermo. MSX is not yet known to be transmitted oyster to oyster but lacking clear evidence of the exact means of transmission, it still seems prudent to discourage oyster movement throughout the area.

Acknowledgment

Testing of Great Bay system oysters has been a team effort. Others involved besides NHF&G include UNH, Jackson Estuarine laboratory personnel, Spinney Creek Shellfish, Inc. and the analytical laboratories at the University of Maine and Rutgers-Haskin Shellfish Research Laboratory. This report has been prepared by NHF&G and we assume all responsibility for its accuracy. To all others on the team we extend our gratitude for their cooperation.

References

Barber, B. J., R. Langan and T.C. Howell, 1997 *Haplosporidium nelsoni (MSX) Epizootic in the Piscataqua River Estuary*. Jour. Parasitol. Vol 83 No. 1, Feb. 1997.

Haskin, H.H. and J. D. Andrews, 1988 *Uncertainties and speculations about the life cycle of the eastern oyster pathogen Haplosporidium nelsoni (MSX) In: W. S. Fisher (ed) Disease Processes in Marine Bivalve Mollusca*, American Fisheries Society, Bethesda, MD. pp. 5-22.

Krantz, G. E., L. R. Buchanan, C. A. Farley, and H.A. Carr, 1972 *Minchinia Nelsoni in Oysters from Massachusetts Waters*, Proceedings of the National Shell Fisheries Association. Vol. 62, June 1972.

Sindermann, C.J. and A Rosenfield, 1967. *Principal Diseases of Commercially Important Marine Bivalve Mollusca and Crustacea*, U. S. Fish and Wildlife Service. Fish Bulletin 66:335-385